New and Notable

Signaling Crosstalk: New Insights Require New Vocabulary

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Signaling complexity is the nightmare of every systems biologist. Some hope that the pathway of interest can be considered in isolation, or else pursue their slightly more accessible bacterial cousins. Others dive into the complexity of mammalian signaling pathways, at least within the safety of mathematical modeling. This is what Rowland et al. (1) did with a focus on crosstalk between pathways, published in this issue of the *Biophysical Journal*.

The problem researchers face is that the human genome encodes hundreds of different phosphatases and kinases. To make things worse, kinases can have many different targets. For instance, Akt and receptor tyrosine kinases of the EGF/ErbB family can selectively interact with over a dozen substrates each. And while there are fewer phosphatases than kinases, they are also more promiscuous or use adaptor proteins to act upon many different substrates. As some of these pathways lead to strong amplification of incoming signals, they are popular targets of pharmaceutical inhibitors. However, due to the crosstalk, predicting an inhibitor' specific effects is rendered difficult.

The basic foundation of the work by Rowland et al. sits on the futile cycle of a kinase and a phosphatase acting upon a substrate. Goldbeter and Koshland (2) established in the 1980s that when these antagonizing enzymes work at saturation, i.e., with zero-order kinetics independent of the substrate concentration, the result is ultra-high sensitivity. If the forward reaction rate, e.g., due to a stimulus, is minimally larger than the reverse reaction rate, all substrate becomes phosphorylated in an all-or-none fashion. The opposite is true when the reverse reaction slightly dominates. In reality, however, these cycles occur within larger pathways, with the mitogen-activated protein kinase cascade for cell growth a classical example. These and similar pathways can have multiple useful functions for the cell (3), including heightened sensitivity to stimuli via amplification along the cascade (4). Alternatively, cascades can produce oscillations and reduce noise with negative feedback from the end to the beginning of the cascade. Or cascades can make bistable switches and exhibit hysteresis with positive feedback from the beginning to the end of the cascade (5). Substrates can also have many phosphorylation sites, e.g., as occurs for the enzymes involved in pheromone sensing in budding yeast (6) or the p53 transcription factor in humans (7).

However, what about the pressing issue of understanding crosstalk between pathways under physiological conditions? Rowland et al. systematically started from a simple pathway with two substrates, where a single kinase and a single phosphatase are shared. Each substrate acts as a competitive inhibitor for the modification of the other substrate, as both substrates compete for the same enzymes. As a result, an ultrasensitive response of one substrate is transferred to the other substrate, even if it does not saturate the enzymes itself. With many substrates available, the entire system can show ultrasensitivity even when none of the substrates saturate the kinase and phosphatase individually.

What if only the kinase or phosphatase is shared? Consider two substrates. For two individual phosphatases and a shared kinase, the saturating substrate can act as a type of gatekeeper and impose a response threshold on the other substrate (see also Harrington et al. (8)). In contrast, for two individual kinases and a shared phosphatase, the saturating substrate essentially enslaves the other substrate in responding ultrasensitively as well. From here, the authors moved on to cascades in which the phosphorylated substrates act as the kinase of the next substrate.

If the phosphatase is shared for all steps of the cascade, the outcome is an even greater sensitivity. This arises because the upstream kinase not only produces more phosphorylated substrate at each step but the phosphorylated substrates also, collectively, inhibit the shared phosphatase. Rowland et al. introduce the term "phosphatase tunneling" for this effect, another example of the new vocabulary required by the subtle mechanisms they have uncovered. Such intuitive vocabulary has a long tradition is physics-based modeling. Famously, "quasi-horse" was humorously introduced to describe the many-body effect of a horse with its surrounding dust particles and flies (9).

Due to the experimental complexity, no direct comparison with data is yet available, although some progress has been made using inverse modeling. In this context, the study by Sachs et al. (10) using Bayesian inference and multicolor flow cytometry comes to mind. However, even in absence of actual data, the article teaches awe of the complexity of real signaling cascades, as well as mindfulness when developing pharmaceutical inhibitors for drug applications. As the authors show, inhibitors can have multiple counterintuitive effects. For instance, inhibiting the phosphorylation reaction between a kinase and one particular substrate can lead to the increased responsiveness and phosphorylation of another substrate if the kinase is shared. Hence, to avoid turning signaling cascades

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into wild hornets' nests or tricky spider webs, the full network architecture needs to be deciphered when trying to make rational predictions.

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